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(71) Applicant *(for all designated States except US)*: UNIVERSITY COLLEGE LONDON [GB/GB]; 5 Gower Street, London WC1E 6HA (GB).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)* : ROOK, Graham, Arthur, William [GB/GB]; Old Hall, Old Hall Road, Steeple Bumpstead, Haver Hill, Suffolk CB9 7EJ (GB). STANFORD, John, Lawson [GB/GB]; Millhouse, Claygate, Marden, Kent. TN12 9TE (GB).

(74) Agents: COLLIER, Jeremy, Austin, Grey et al.; J.A. Kemp & Co., 14 South Square, Gray's Inn, London WC1R 5LX (GB).

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With international search report.

(54) Title: MYCOBACTERIUM VACCAE IN THE TREATMENT OF UVEITIS

(57) Abstract

Antigenic and/or immunoregulatory material derived from *Mycobacterium vaccae* is useful in the treatment of uveitis.

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+ DESIGNATIONS OF "SU"

Any designation of "SU" has effect in the Russian Federation. It is not yet known whether any such designation has effect in other States of the former Soviet Union.

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Mycobacterium vaccae in the treatment of uveitis

This invention relates to the treatment of uveitis.

British Specification No. 2156673 describes immunotherapeutic agents comprising killed cells of Mycobacterium vaccae. These agents are useful in the immunotherapy of mycobacterial disease, especially tuberculosis and leprosy. It is stated that use of this immunotherapeutic agent facilitates the removal of the persisting bacilli responsible for tuberculosis or leprosy which, as is well known, it is difficult to remove by chemotherapy alone. It is suggested in the specification that the immunotherapeutic agent is believed to act by presenting the "protective" common mycobacterial antigens to advantage and by containing immune suppressor determinants which are active in regulating disadvantageous immune mechanisms. As a consequence, "persister" bacilli are recognized by the immune system by their content of common mycobacterial antigens and effective immune mechanisms are directed against them, in the absence of the tissue necrotic form of immunity usually present in mycobacterial disease.

International Patent Specification PCT/GB 85/00183 describes compositions for the alleviation of the symptoms of, and for the treatment or diagnosis of, arthritic diseases which comprise as active ingredient the whole organism of M. vaccae. It is stated that the preparations

of M. vaccae are useful for the treatment of various autoimmune diseases and especially arthritic conditions including rheumatoid arthritis, ankylosing spondylitis or Reiter's syndrome.

Uveitis is a condition, often observed in leprosy patients but also found in other individuals, which is difficult to treat and leads to permanent blindness. The present invention is founded upon the surprising observation that compositions comprising antigenic and immunoregulatory material derived from Mycobacterium vaccae are useful in the treatment of uveitis.

The present invention accordingly provides a method for the treatment of uveitis which comprises administering to the patient suffering from such a condition an effective amount of a therapeutic composition comprising antigenic and immunoregulatory material derived from Mycobacterium vaccae.

The invention further provides antigenic and immunoregulatory material derived from M. vaccae for use in the manufacture of a therapeutic agent for the treatment of uveitis. Such antigenic and immunoregulatory material is also provided for use in the manufacture of a therapeutic agent for use in the treatment of uveitis.

The therapeutic agent of the invention conveniently, and therefore preferably, comprises dead cells of M. vaccae, most preferably cells which have been

killed by autoclaving or by irradiation. The therapeutic agent normally comprises more than 10^8 microorganisms per ml of diluent, and preferably from 10^8 to 10^{11} killed M. vaccae microorganisms per ml of diluent.

The diluent may be pyrogen-free saline for injection alone, or a borate buffer of pH 8.0. The diluent should be sterile. A suitable borate buffer is:

$Na_2B_4O_7.10H_2O$	3.63 g
H_3BO_3	5.25 g
NaCl	6.19 g
Tween 80	0.0005%
Distilled Water	to 1 litre

The preferred strain of M. vaccae is one denoted R877R isolated from mud samples from the Lango district of Central Uganda (J.L. Stanford and R.C. Paul, Ann. Soc. Belge Med, Trop. 1973, 53 141-389). The strain is a stable rough variant and belongs to the aurum sub-species. It can be identified as belonging to M. vaccae by biochemical and antigenic criteria (R. Bonicke, S.E. Juhasz., Zentr albl. Bakteriol. Parasitenkd. Infection skr. Hyg. Abt. 1, Orig., 1964, 192, 133).

The strain denoted R877R has been deposited under the Budapest Convention at the National Collection of Type Cultures (NCTC) Central Public Health Laboratory, Colindale

Avenue, London NW9 5HT, Unit d Kingdom on February 13th, 1984 under the number NCTC 11659.

For the preparation of the therapeutic agent, the microorganism M. vaccae may be grown on a suitable solid medium. A modified Sauton's liquid medium is preferred (S.V. Boyden and E. Sorkin., J. Immunol, 1955 75, 15) solidified with agar. Preferably the solid medium contains 1.3% agar. The medium inoculated with the microorganisms is incubated aerobically to enable growth of the microoganisms to take place, generally at 32°C for 10 days. The organisms are harvested, then weighed and suspended in a diluent. The diluent may be unbuffered saline but is preferably borate-buffered and contains a surfactant such as Tween 80 as described above. The suspension is diluted to give 100 mg of microorganism/ml. For further dilution, borate buffered saline is preferably used so that the suspension contains 10 mg wet weight of microorganisms/ml of diluent. The suspension may then be dispensed into 5 ml multidose vials. Although the microorganisms in the vials may be killed using irradiation e.g. from ^{60}Cobalt at a dose of 2.5 megarads, or by any other means, for example chemically, it is preferred to kill the microorganisms by autoclaving, for example at 10 psi (69 kPa) for 10 minutes (115°-125°C). It has been discovered, unexpectedly, that autoclaving yields a more effective preparation than irradiation.

The therapeutic agent is in general administered by injection in a volume in the range 0.1-0.2 ml, preferably 0.1 ml, given intradermally. A single dosage will generally contain from 10^7 to 10^{10} killed M. vaccae microorganisms. It is preferred to administer to patients a single dose containing 10^8 to 10^9 killed M. vaccae. However, the dose may be repeated depending on the condition of the patient.

While the present invention does not depend on the truth of this theory it is believed that the active ingredient in the killed M. vaccae may be the 65 kDa mycobacterial heat shock protein (hsp 65) described by Young et al. "Stress proteins are immune targets in leprosy and tuberculosis", Proc. Natl. Acad. Sci. U.S.A. 85 (1988), pp4267-4270 in a form obtained from M. bovis. The preferred autoclaved M. vaccae cells used in the present invention are believed to provide an effective package of the hsp 65 and other substances in a convenient adjuvant.

Although the therapeutic agent will generally be administered by intradermal injection, other routes, e.g. oral administration, can also be used.

It may be advantageous and is within the scope of the invention to use more than one strain of M. vaccae, and/or to include in the immunoprophylactic agent other mycobacterial antigens. Tuberculin may also be included.

The immunoprophylactic agent may also contain BCG

(Bacillus Calmette-Guerin) vaccine, in particular the freeze-dried form of the vaccine, to promote its effect.

The therapeutic agent can contain further ingredients such as adjuvants, preservatives, stabilisers etc. It may be supplied in sterile injectable liquid form or in sterile freeze-dried form which is reconstituted prior to use.

M. vaccae may be used as such or as an extract or fractioned portion of the organism to manufacture the therapeutic agents according to the invention.

The following Example illustrates the invention.

EXAMPLE

M. vaccae NCTC 11659 is grown on a solid medium comprising modified Sauton's medium solidified with 1.3% agar. The medium is inoculated with the microorganism and incubated for 10 days at 32°C to enable growth of the microorganism to take place. The microorganisms are then harvested by gently scraping the surface of the agar and weighed (without drying) and suspended in M/15 borate buffered saline at pH8 to give 10 mg of microorganisms/ml of saline. The suspension is dispensed into 5 ml vials, and then autoclaved for 10 minutes at 10 psi (69 kPa) to kill the microorganisms. After cooling, the therapeutic agent thus produced is stored at 4°C before use. A single dose consists of 0.1 ml of the suspension, which should be shaken vigorously immediately before use, containing 1 mg

wet weight of M. vaccae. The dose is given by intradermal injection n rmally over the left deltoid muscle.

Of 148 fully treated leprosy patients, 79 were given M. vaccae therapy and 69 received a placebo. In the group receiving M. vaccae therapy, 17 showed symptoms of uveitis and of these, 13 were cleared of uveitis one year after therapy. In contrast, of the 69 patients receiving placebo, 12 showed symptoms of uveitis at the start of treatment and the uveitis cleared in only 4. This result is significant at $p<0.005$.

CLAIMS

1. Use of antigenic and/or immunoregulatory material derived from Mycobacterium vaccae in the manufacture of a therapeutic agent for the treatment of uveitis.

2. The use according to claim 1, wherein the antigenic and/or immunoregulatory material derived from M. vaccae comprises dead cells of M. vaccae.

3. The use according to claim 2, wherein the cells of M. vaccae have been killed by autoclaving.

4. The use according to claim 1, wherein the antigenic and/or immunoregulatory material derived for M. vaccae comprises the 65 kDa heat shock protein.

5. The use according to any one of the preceding claims, wherein the material derived from M. vaccae is derived from the strain as deposited at the National Collection of Type Cultures (NCTC) Central Public Health Laboratory, Colindale Avenue, London NW9 5HT, United Kingdom on February 13th, 1984 under the number NCTC 11659.

6. The use according to any one of the preceding claims, wherein the therapeutic agent contains, per dose, antigenic and/or immunoregulatory material from 10^7 to 10^{10} M. vaccae microorganisms.

7. A method for the treatment of uveitis which comprises administering to the patient suffering from such a condition an effective amount of antigenic and/or

immunoregulatory material derived from Mycobacterium vaccae.

8. A method according to claim 7, wherein the material derived from M. vaccae is as defined in any one of claims 2 to 6.

9. Products comprising antigenic and/or immunoregulatory material derived from Mycobacterium vaccae for use in treatment of uveitis.

10. Products according to claim 9, wherein the material derived from M. vaccae is as defined in any one of claims 2 to 6.

11. A pharmaceutical agent for use in the treatment of uveitis which agent comprises antigenic and/or immunoregulatory material derived from Mycobacterium vaccae.

12. An agent according to claim 11, wherein the material derived from M. vaccae is as defined in any one of claims 2 to 6.

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 91/01970

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl.5 A 61 K 39/04

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl.5	A 61 K · C 07 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
X	WO,A,8503639 (UNIVERSITY COLLEGE LONDON) 29 August 1985, see the whole document	9-12
X	WO,A,8505034 (UNIVERSITY COLLEGE LONDON) 21 November 1985, see the whole document	9-12
X,P	WO,A,9102542 (UNIVERSITY COLLEGE LONDON) 7 March 1991, see the whole document	9-12
A	EP,A,0262710 (DE STAAT DER NEDERLANDEN) 6 April 1988, see the whole document	4
A	Proceedings of the National Academy of Sciences, volume 85, June 1988, Biochemistry (Washington DC,US) D. Young et al.: "Stress proteins are immune targets in leprosy and tuberculosis", pages 4267-4270, see the whole article (cited in the application)	4

° Special categories of cited documents:[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
17-01-1992	12. 02. 92
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	Maria Peis Maria Peis

Form PCT/ISA/210 (second sheet) (January 1985)

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. [X] OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. [ ] Claim numbers _____, because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 7 - 8 are directed to a method of treatment of the human body the search has been carried out and based on the alleged effects of the composition.

2. [ ] Claim numbers _____, because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claim numbers _____, because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. [ ] OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. [ ] As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

[ ] The additional search fees were accompanied by applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. GB 9101970 SA 53079

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 04/02/92
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A- 8503639	29-08-85	AU-A- 3938885	10-09-85
		EP-A- 0172212	26-02-86
		GB-A,B 2156673	16-10-85
		US-A- 4724144	09-02-88
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EP-A- 0262710	06-04-88	NL-A- 8602270	05-04-88
		NL-A- 8701163	05-04-88
		AU-B- 601765	20-09-90
		AU-A- 7800087	17-03-88
		JP-A- 63126895	30-05-88
		ZA-A- 8706738	14-03-88